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Reproductive Performance of Holstein-Friesian Dairy Cows Subjected to Different Treatments for Cystic Ovarian Follicles

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ABSTRACT

Cystic ovarian follicles "COF" is a common ovarian disorder diagnosed in dairy cattle that cause significant economic losses in dairy industry. This study was designed to evaluate efficacy of different therapeutic protocols of COF without determining cyst type. The study was conducted on 104 Holstein-Friesian cystic cows that were divided randomly to the following treatments: *Experiment I:* 1) Group G, n=27, subdivided into A) n=13: treated with 20µg Buserelin-GnRH agonist and B) n=14: treated with 100µg Gonadorelin-GnRH agonist; 2) Group GP, n=23: received 20µg Buserelin on day 0- 500µg of PGF2α on day 10; 3) Group GGP, n=27: received 2 doses of 20µg Buserelin 7 ds apart followed by 500µg PGF2α 7ds from the 2nd Buserelin injection; 4) Group GPG (Ovsynch), n=17: received 20µg Buserelin on day 0- 500µg PGF2α on day 7, and 48hrs later, 20µg Buserelin was injected; 5) Group C, n=10: no treatment received. 22 normal cycling cows were kept as a normal control-NC. *Experiment II:* This part of the study was conducted on 34 cystic animals that did not respond to one of the previous treatments in experiment I and were divided into 2 groups: 1) Group CGP, n=19: CIDR+100µg Gonadorelin- (d 0), then 500µg of PGF2α on day 7, insert was removed on day 9; 2) Group PI, n=15: treated by oral administration of 5gm Potassium iodide dissolved in 500 ml sterile water for 7 successive days. Treated Cows were observed for 35 days from the end of treatment, females exhibited estrus were inseminated within 12 hours of detected heat. Pregnancy was confirmed by per rectum on day 50 post-insemination. Results showed that GP protocol achieved highest (P<0.05) estrus induction rate (EIR) recorded at 87%, followed by 70% for G and GGP group, and lowest (53%) obtained in GPG treated cows. Conception rate at 1st estrus and total conception rate (3 cycles after treatment) did not differ significantly among treatment groups, while pregnancy rates (PR) were significantly different (P<0.05) with highest (65.21%) obtained in GP protocol. Other groups recorded 63%, 55.55%, and 41.17% PR for G, GGP, and GPG respectively. In experiment II, EIR was 47.36% and 33.33% for CGP and PI respectively with no significant difference, all conceived after 3 estrous cycles by the end of the experiment. In conclusion, GnRH+ PGF2α 10ds later was the most suitable treatment of COF regardless of cyst type, GGP protocol might be a profitable alternative to Ovsynch for treatment of cystic ovaries. Persistent cases of COF could be recruited to avoid culling for other treatment options such as CIDR or Potassium iodide with equal therapeutic efficacy.

1. INTRODUCTION

Cystic ovarian follicles "COF" was defined as anovulatory follicles (<2 cm) that fail to regress and continue to grow, secrete steroids, and interfere with normal ovarian cyclicity (Vanholder et al., 2006). Others defined COF as follicular structures having a diameter 17-25 mm that persist for more than 6 days in the absence of corpus luteum "CL" (Silvia et al., 2002; Youngquist and Threlfall, 2007). Transrectal ultrasonography have shown that some COF could be purely follicular that secrete high levels of estradiol, or partially luteinized usually called "luteal cyst" that produce significant levels of progesterone (Douthwaite and Dobson, 2000). Under field conditions, it is hard to distinguish

between follicular and luteal cyst because accurate diagnosis needs both ultrasonography and assessment of progesterone (P4) in blood. Some risk factors have been associated with cystic ovaries, such as heredity, cow parity, seasonal variation, high milk production, abnormal puerperium, uterine infection, and nutrition (Arthur, 2001; Kirk et al., 1982; Lopez-Gatius et al., 2002; Peter, 2004; Zulu et al., 2002).

According to literature, the most widely accepted cause of COF is a neuroendocrine imbalance affecting the hypothalamic-hypophyseal-gonadal axis. The pre-ovulatory surge of luteinizing hormone (LH) is either blocked, insufficient in magnitude or mistimed during dominant follicle maturation; anovulation and cyst formation is the result (Vanholder et al., 2006). Other pathogenic pathways related to ovarian factors have been also suggested; alterations in expression of LH, FSH receptors (Kawate et al., 1990), and estradiol receptor β (Odore et al., 1999) was noted in granulosa cells of cysts compared to normal follicles. COF is associated with great losses to dairy business, it leads to longer calving-calving intervals, delayed postpartum conception, increased number of inseminations per conception (Hooijer et al., 2001), and increased culling rate (Grohn et al., 1990). Treatment of COF is challenging as some cases either seem to be recurrent or resistant to treatment. Therapeutic protocols are similar for both types of COF, gonadotropin releasing hormone "GnRH", human chorionic gonadotrophin "hCG" (Mollo et al., 2012; Nakao et al., 1992), with (Bartolome et al., 2000; Lopez-Gatius et al., 2001) or without PGF2α (Probo et al., 2011) have been used with variable efficacy. Progesterone-releasing intravaginal devices have been also used (Douthwaite and Dobson, 2000; Lopez-Gatius et al., 2001). The objective of the current study was: 1) to determine the most effective therapeutic protocol for COF in absence of information about cyst type and to relate it with reproductive performance of treated cows, 2) investigate ability of CIDR and Potassium iodide to resolve persistent cases of COF before being categorized as culled animals.

2. MATERIALS AND METHODS

2.1. Animals:

The present study was performed over the period from September 2013 to February 2016 in a farm located at Dakahlia Governorate, Egypt. The animals used for the experiment were Holstein-Friesian cows with good health condition weighing 400-450 kg of 3±0.5 body score condition. Cows were fed on optimized total mixed ration, received regular deworming medicines and vaccinated against common infectious diseases. Cows were kept in open yards, milked mechanically 3 times a day and were dried 70 days before the expected date of parturition. The herd was under close veterinarian supervision to monitor general and reproductive health condition.

2.2. Experimental design:

Cows with COF were diagnosed during the postpartum routine rectal examination between days 45-60 days after parturition. Rectal finding characterized by presence of fluid-filled structure, with a diameter of at least 2.5 cm that persisted for more than 10 days, with absence of corpus luteum and absence of uterine tonicity. Parity of cystic cows ranged from 2- 5 lactational season, their milk yield was either 15-25kg (n=30) or >25kg (n=74) at day of diagnosis. The study was performed over 2 experiments as follow:

2.3. A. Experiment I:

104 diagnosed with COF were assigned randomly into 5 groups according to treatments:

1) Group **G**, n=27subdivided into:

A) n=13: received I.M injection of 20µg Buserelin-GnRH agonist; Receptal® (Intervet / Schering-Plough animal health)

B) n=14: treated with 100µg Gonadorelin-GnRH agonist; Cystorelin® (Ceva Sante Animale, France).

2) Group **GP**, n=23: received 20µg Buserelin followed 10 days later by 500µg of Cloprostenol-PGF2α analogue; Estrumate® (Intervet/Schering-Plough Animal Health).

3) Group **GGP**, n=27: received 2 doses of 20µg Buserelin 7 days apart followed by 500µg PGF2α 7days from the 2nd Buserelin injection.

4) Group **GPG**, n=17: received 20µg Buserelin followed 7 days later by 500µg PGF2α and another dose of 20µg Buserelin 48hrs from PGF2α (Ovsynch).

5) Group **C**, n=10: served as control animals with no treatment received.

22 normal cycling cows were kept as a normal control (N C) group.

2.4. B. Experiment II:

This experiment was conducted on 34 cows that did not respond to one of the previous treatments in experiment I, and were divided into 2 groups:

1) Group **CGP**, n=19: received on day 0 intravaginal progesterone insert CIDR® (Eazi-Breed insert containing 1.38 g of P4, Pfizer Animal Health) +100µg Gonadorelin, then 500µg of PGF2α on day 7, insert was removed on day 9.

2) Group **PI,** n=15: treated by oral administration of 5gm Potassium iodide (El-Gomhouria Co for chemicals, Egypt) dissolved in 500 ml sterile water for 7 successive days.

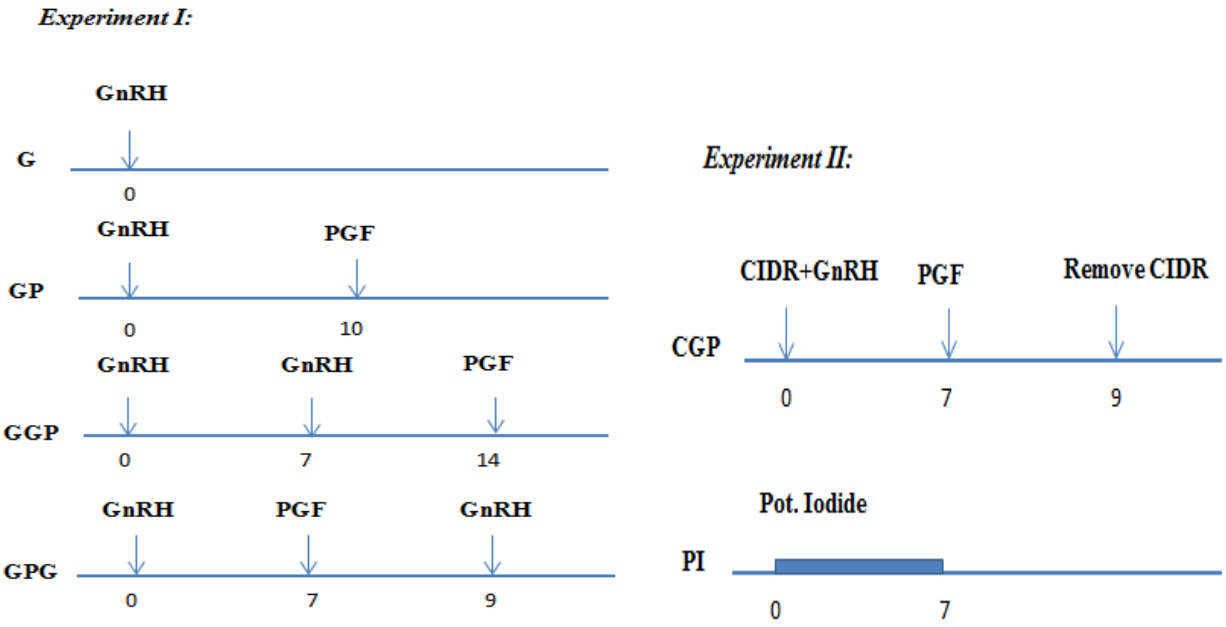

Figure 1: Schematic diagram of therapeutic protocols employed in the study.

2.5. Detection of estrus, insemination and pregnancy diagnosis:

Treated cows were observed 3 times a day for estrus detection by trained persons within 35 days from end of treatment; heat was confirmed by rectal examination. Cows in estrus with normal genital tract (tonic uterus and cervical mucus) were artificially inseminated within 12 hours after exhibiting heat signs by the same skilled veterinarian. Pregnancy was diagnosed per rectum at 50 days after AI, and the following reproductive parameters were recorded: estrus induction rate (EIR), treatment estrus-interval, number of services per conception, conception rates (percentage of conceived cows out of total females inseminated), interval from treatment to first conception, and pregnancy rate (PR) (percent of cows exhibited estrus multiplied by total conception rate)/100 (Bartolome et al., 2000) by the end of the experiment, which was limited by 3 estrous cycles after treatment.

2.6. Statistical analysis:

All frequencies were analyzed by chi-square test by the aid of SAS (SAS, 2002). Analysis of variance (ANOVA) was conducted to assess the significance between treatment groups more than two, while two groups were analyzed by T-independent test. Differences at $P<0.05$ were considered significant.

3. RESULTS

3.1. Experiment I:

Data on distribution of estrus exhibition, estrus induction rate, and treatment-estrus interval are presented in table 1. Data on group G (Buserelin and Gonadorelin-treated animals) was pooled as there was no significant difference between them. Results indicated that the highest EIR (87%) was obtained in GP group, while it was (70%) for both G and GGP groups. The lowest (53%) EIR after treatment was observed in GPG protocol, only (30%) of animals spontaneously recovered in the control group. Estrus was synchronous in GP and GGP groups where the highest percent of animals exhibited estrous in the 3rd week after treatment, while in GPG group most of cows (35.3%) showed estrus in the 2nd week. Estrus was least synchronous in group G; cows continued exhibiting estrus over the whole observation period (35 days after treatment). Mean days to 1st estrus did not differ significantly among treatment groups, and ranged between 14.22±2.33 to 19.21±1.26 days from end of treatment.

Table 2 shows reproductive performance represented by number of services per conception, treatment-conception interval, and conception/pregnancy rates of 3 inseminations after treatment. Results indicated that mean days from treatment to conception was 25.35±4.92 and 26.53±3.73 for G and GGP groups respectively,

while it was delayed for GPG and GP groups (33.86±6.51 Vs 35.40±5.67) without significant difference to the previous values. Highest number of services per conception (1.86±0.26) was recoded for GPG group, while it did not differ significantly among other treated group. Conception rate at 1st estrus and total conception rate did not differ significantly among treatment groups. Pregnancy rate was significantly higher in GP group (65.21%) in comparison to control and other treatment protocols (P<0.05), and was recorded at 63%, 55.55%, and 41.17% for G, GGP, and GPG respectively.

3.2. Experiment II:

Table 3 shows frequency of estrus exhibition, EIR, and treatment-estrus interval of cystic cows subjected to treatment with CIDR or Pot. iodide. Results indicated that EIR did not differ significantly between the 2 groups, however treatment-estrus interval was significantly shorter in CGP group recorded at 10.55±0.83 versus 23.8±4.36 for PI group (P<0.01). Fertility parameters did not differ significantly (P>0.05) between the 2 groups as shown in table 4.

Table 1: Frequency of estrus exhibition, EIR, and treatment-estrus interval of cystic cows after different treatments in experiment I:

Groups	N	Frequency of estrus exhibition from end of Treatment in days N (%)				Overall EIR* N	%	Mean days to 1st estrus Mean ± SE
		T-7	8-15	16-21	22-35			
C	10	0.0	0.0	0.0	3(30)	3	30.0	26.00±2.08a
G	27	3(11.1)	3(11.1)	8 (29.6)	5(18.5)	19	70.3	15.37±1.79b
GP	23	0.0	5(21.7)	13(56.5)	2(8.7)	20	87.0	17.90±1.76b
GGP	27	0.0	0.0	15(55.5)	4(14.8)	19	70.3	19.21±1.26ab
GPG	17	0.0	6(35.3)	1(5.9)	2(11.7)	9	53.0	14.22±2.33b
Total	104	3	14	37	16	70	67.3	

* EIR: estrus induction rate values are significant at (P< 0.05).
Means bearing different letters are significant at (P<0.05)

Table 2: Fertility parameters after insemination of treated cows exhibited estrus in experiment I:

Groups	No of Cows insem.	Treatment-Con.interval Mean ± SE	No of S/C Mean ± SE	Conception rate/estrus cycle N (%)			Total CR N (%)	Pregnancy Rate %*
				1st	2nd	3rd		
C	3	--	1.00±0.00b	2 (66.6)	-	-	2 (66.66)	20
N C	22	--	1.61±0.16ab	9 (40.9)	7 (31.8)	2 (9.1)	18 (81.81)	81.81
G	19	25.35±4.92a	1.29±0.14ab	13 (68.4)	3 (15.7)	1 (5.26)	17 (89.47)	63
GP	20	35.40±5.67a	1.27±0.15ab	9 (45)	2 (10)	4 (20)	15 (75.00)	65.21
GGP	19	26.53±3.73a	1.67±0.23ab	12 (63.1)	2 (10.5)	1 (5.26)	15 (78.94)	55.55
GPG	9	33.86±6.51a	1.86±0.26a	2 (22.2)	4 (44.44)	1 (11.1)	7 (77.77)	41.17

Means bearing different letters are significant at (P<0.05)
* Pregnancy rates values are significant at (P< 0.05).

Table 3: Frequency of estrus exhibition, EIR, and treatment-estrus interval of cystic cows after different treatments in experiment II:

Groups		Frequency of estrus exhibition from end of Treatment in days N (%)				Overall EIR		Mean days to 1st estrus Mean ± SE
						N	%	
	N	**T-7**	**8-15**	**16-21**	**22-35**			
CGP	19	0.0	8(42.1)	1(5.26)	0.0	9	47.36	10.55±0.83b
PI	15	0.0	1(66.6)	1(66.6)	3(20)	5	33.33	23.80±4.36a

Means bearing different letters are significant at (P<0.01)

Table 4: Fertility parameters after insemination of treated cows exhibited estrus in experiment II:

Groups	No of Cows insem.	Treatment-Con.interval Mean ± SE	No of S/C Mean ± SE	Conception rate/estrus cycle N (%)			Total CR N (%)	Pregnancy Rate %
				1st	2nd	3rd		
CGP	9	33.44±8.06	1.89±0.31	4 (44.4)	2 (22.2)	3 (33.3)	9 (100)	47.36%
PI	5	31.80±8.48	1.40±0.24	3 (60)	2 (40)	0	5 (100)	33.33%

4. DISCUSSION

A lot of efforts have been employed to treat cystic follicles in attempts to control financial losses associated with this disease. Our study employed 4 standard GnRH-based treatment protocols intentionally used irrespective of cyst type to mimic farm conditions that might lack diagnostic tools such as ultrasonography and hormonal assays. On day of diagnosis of cystic cows, 2 groups of milk yield were found: 15-25kg (n=30) representing 28.8% or >25kg (n=74) representing 71.15%, which support the positive correlation between milk yield and incidence of COF (Hooijer et al., 2001; Lopez-Gatius et al., 2002). This correlation may be due to genetic reasons or stress of lactation, which results in metabolic/hormonal disturbances that may be the reason or contributing reason for COF (Arthur, 2001).

Results of the current study showed that GP group achieved highest EIR recorded at 87% with 17.9±1.76 days as treatment-estrus interval, 13 out of 23 animals (56.5%) in GP group exhibited estrus signs in the 3rd week after treatment (table 1). Using the same protocol, Saad reported 66.6% EIR (Saad, 2013), while using PGF2α 14 days after GnRH, Gatius and colleagues reported 29.4 % EIR (Lopez-Gatius et al., 2001) and 42 % in a later study (Lopez-Gatius and Lopez-Bejar, 2002). Using GnRH-7ds-PGF2α regimen, 34.9% EIR was obtained (Bartolome et al., 2000); it is obvious from our results that GnRH-10ds-PGF2α is more effective therapeutic program. During GP treatment, regression of luteinized cyst that resulted from GnRH treatment is induced 7-14 days later with exogenous PGF2α. The result is shortening the interval from treatment to estrus and an increase in the degree of estrus synchrony.

GnRH alone resulted in 70% EIR, which is in agreement with results of probo et al, reporting 71.3% EIR (Probo et al., 2011), and within ranges (64- 82%) recorded by Bierschwal et al after GnRH injection in different doses (Bierschwal et al., 1975). Our result is higher than that obtained by molo et al recorded at 64% (Mollo et al., 2012), and 48% (Saad, 2013) within 30 ds of treatment. while it was 37.1% (Kim et al., 2006), and 28% (Dinsmore et al., 1990) within 21 ds after treatment. Estrus in G group was least synchronous presumably owing to the status of the ovary at time of treatment. GnRH was effectively used to resolve both types of cystic ovaries, i.e. follicular and luteal cyst. Its mode of action has been explained into 2 ways: GnRH either induces luteinization of the cyst or promotes ovulation and the formation of new corpora lutea. However, it was shown that GnRH injection induced ovulation of follicle other than cystic one that was present at time of treatment (Ambrose et al., 2004).

Similar to single GnRH injection, treatment with GGP protocol resulted in 70% EIR, which in agreement with results obtained previously (Shaker, 2013), who reported 70.24% induction rate using the same protocol for cystic cows. When compared to each other, GPG (Ovsynch protocol) was less efficient than GGP in terms of recovery rate (53% for GPG Vs 70% for GGP). GGP protocol resemble partially a newly developed pre-synch regimen that uses PGF2α-2d-GnRH 6 ds "G6G" or 7ds "G7G" before an Ovsynch program, results are higher ovulations and pregnancy rates (Bello et al., 2006; Dirandeh et al., 2015). Apparently, GGP protocol allowed enough time (14 ds) for both follicular and luteal cysts to resolve and ensure the presence of luteinized tissue at time of PGF2α injection. Mean days to 1st estrus did not differ significantly among treated groups, and ranged between 14.22±2.33 to 19.21±1.26 ds from end of treatment.

Results on fertility parameters as depicted in table 2 showed that treatment-conception interval, number of services per conception, conception rate at 1st estrus, and total conception rate (3 estrus cycles after treatment) did not differ significantly among treatment groups. However, pregnancy rate (PR) was significantly higher in GP group (65.21%) in comparison to other treatment protocols that was recorded at 63%, 55.55%, and 41.17% for G, GGP, and GPG respectively. In their study, Taktaz and colleagues obtained higher PR recorded at 76.6% using the same GP protocol (Taktaz et al., 2015). Lower rates were recorded in other studies of about 18% (Bartolome et al., 2000), 13% (Lopez-Gatius and Lopez-Bejar, 2002), and 5.9% (Lopez-Gatius et al., 2001). In the 2nd order after GP treatment, GnRH alone was able to achieve 63% PR, which is within ranges (46.42 %-71.42%) reported before (Bierschwal et al., 1975). While, higher value of 71.8% was obtained by Dinsmore and colleagues (Dinsmore et al., 1990). PR of about 20% was reported by molo et al in cystic Friesian cows using single injection of GnRH (Mollo et al., 2012)

Presently, there are no studies providing data on conception/pregnancy rates in response to GGP in cystic cows. Herein we report 55.55% PR (15 out of 27) in cystic cows t
reated with GGP regimen with 1.67±0.23 as number of services per conception, which was statistically comparable to the normal control group (table 2). In the current study, compared to GGP group, Ovsynch or GPG program achieved lower PR recorded at 41.17%, which is similar to 41% obtained by Ambrose and co-workers (Ambrose et al., 2004). Also Ozturk and co-workers obtained 29.8% (Ozturk et al., 2010), 46.7% (Amr and Badr, 2007), while rate of about 26.9% was reported in other studies (Bartolome et al., 2000; Fricke and Wiltbank, 1999). From an economic point of view, GGP protocol might be a good alternative to Ovsynch for treatment of cystic ovaries according to our results (70% Vs 53% EIR, and 55.55% Vs 41.17 PR, respectively).

In this part of our study, 34 cows that resisted one of the previous therapeutic protocols in experiment I were subjected to 2 treatments 1) CGP, n=19: received on day 0 CIDR+ GnRH, then PGF2α on day 7, insert was removed on day 9. 2) P.I, n=15: treated orally with 5gm of Potassium iodide for 7 successive days. Results of response as shown in table 3 indicated that, 47.36% EIR was obtained by CGP group and 33.33% for PI that did not differ significantly between the 2 groups. However, treatment-estrus interval was significantly shorter in CGP group recorded at 10.55±0.83 ds versus 23.8±4.36 ds for PI group. Gatius and co-workers reported 83.3% EIR using progesterone-releasing intravaginal device "PRID" for 9ds (Lopez-Gatius et al., 2001), while 100% EIR was recorded after 12 ds PRID (Douthwaite and Dobson, 2000). The low estrus induction rate in experiment II in comparison to other studies might be because cows developed persistent cyst as they received a previous GnRH treatment known to induce LH release. Some previous studies showed that COF is associated with high levels of LH and an increase in its pulse frequency, which favor development and persistence of large cyst (Calder et al., 1999; Taft et al., 1996).

Both treatments employed in experiment II resulted in 100% total conception rate by the end of the experiment (3 estrous cycles after treatment). CGP group attained 47.36% PR (9 pregnant out of 19 total treated), other studies reported 41% (Ambrose et al., 2004), 57.1% (Amr and Badr, 2007) PR using the same CIDR protocol. While it was 50% after 12 ds PRID (Douthwaite and Dobson, 2000), 27.8% after 9 ds PRID (Lopez-Gatius et al., 2001) and 20% when PRID was used for 10 days (Mollo et al., 2012). COF was treated using different doses of potassium iodide in an early study (Dzhambazov et al., 1975), the authors reported 86.6% total conception rate. In the present study, conception rate at 1st estrus was 60% and PR was 33.33% (5 pregnant out of 15 total treated) with 1.40±0.24 as number of services per conception. Potassium iodide has been used to cure different reproductive disorders such as true anestrous (El-Shahat and Badr, 2011; Waheeb and Hatab, 2017), repeat breeder and mucometra (Purohit et al., 2001). It is

being used by different routes; locally via intrauterine infusion for its antiseptic properties, injection, and orally as a source of iodine that have been shown to enhance reproductive performance by supporting thyroid function (Chhabra et al., 2007; Zeedan et al., 2010). Cystic cows were shown to have lower thyroid hormone levels (T4) in comparison to normal cyclic cows (Mutinati et al., 2013).Overall response to therapeutic protocols used in the current study showed that COF can be treated with significant efficacy even in the absence of diagnostic tools for cyst classification. Other studies reported similar results when ultrasonography and P4 assay were used to distinguish cyst type (Probo et al., 2011; Taktaz et al., 2015).

In conclusion, treatment of cystic cows with GnRH followed 10 ds later with PGF2α (GP protocol) recorded highest recovery and pregnancy rate (87% and 65.21% respectively); GGP regimen was superior to Ovsynch (70% Vs 53% EIR, and 55.55% Vs 41.17 PR). To avoid culling, persistent cases of COF could be recruited for other treatment options such as CIDR-GnRH-PGF2α or Potassium iodide with equal therapeutic efficacy.

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